DEVELOPMENT OF A BACTERIAL SKOPOBIOTA FOR THE BIOREMEDIATION OF SEWAGE AND TEXTILE INDUSTRY EFFLUENTS IN YAMUNA RIVER WATER

SYNOPSIS

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INTRODUCTION

Developing nations, such as India, continue to depend on rivers for several activities like irrigation, drinking, transportation, electricity production, bathing, fishing, laundry, recreation, waste cleansing and sewage disposal. Demographic growth along with the increase in agricultural activities and industrialization has led to a continuous increase in the demand for fresh water (Misra, 2010). The accessibility to sufficient quantity of clean water is a serious problem in metropolitan cities. According to World Health Organization, every year 1 million people and 395,000 children under 5 years of age are estimated to die due to the use of contaminated water (World Health Organization, 2023). Consequently, it is important to monitor the change in water quality due to anthropogenic activities.

Yamuna river flows through the cities of Uttaranchal, Uttar Pradesh, Himachal Pradesh, Haryana, Rajasthan, Madhya Pradesh and the union territory Delhi (Malik *et al.,* 2014). Since many decades domestic sewage and industrial waste is being discharged into Yamuna River. This river has been declared as "about to die" by the researchers (Misra, 2010).

About 90% of the wastewater released from households and industries into the river are untreated which affect the quality of Yamuna River water. Thus, making Yamuna River as one of the most polluted rivers in the world (Sharma *et al.,* 2020). According to a news article published by India Today, an appalling amount of polluted water was supplied to 638 villages around Agra region for irrigation, bathing and drinking purposes leading to a large number of health problems among the people using this water.

Several industries like pulp and paper, sugar, tannery, steel plants, distilleries, textiles, leather, chemical, pharmaceuticals, rubber, glass, oil refineries, thermal power plants, foods etc. discharge wastewater in Yamuna River (Sharma and Chaudhry, 2015). Among these industries, solely textile has accounted for 64.2% of the polluting industries discharging effluents into Yamuna (Central Pollution Control Board, 2023). Approximately 80% of the total pollution in Yamuna is caused by domestic sewage essentially from cities such as Sonipat, Panipat, Delhi, Ghaziabad, Mathura, Agra, Etawah and Prayag raj (River Rejuvenation Committee, 2020).

The textile industry effluents and sewage has been recognized as a major contributor of water pollution. Sewage majorly comprises of greywater from sinks, bathtubs, showers, dishwashers and washing machines; blackwater from toilets that consist of human fecal matter, soap and detergents, organic matter

like vegetable and fruit peels, flowers and other waste from temples, fertilizers and herbicides from agricultural runoff, salts, nutrients, oil, grease and paints (Saxena *et al.,* 2020). The textile industry is one of the most chemical-intensive industries. It consumes and discharges large quantities of water every day that are required for wet processes of the industry such as singeing, desizing, kiering and bleaching (Patel and Vashi, 2015). Textile effluents mainly consist of dyes and heavy metals like cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), lead (Pb) and zinc (Zn). Dyes are water pollutants which are difficult to degrade and persist in the environment (Couto, 2009). Heavy metals are present in textile wastewaters due to the use of metal-containing chemicals and dyes in pre-treatment, dyeing, printing and finishing steps (Velusamy *et al.,* 2021). Heavy metals bioaccumulate in tissues and are non-biodegradable due to their complex chemical forms. The existing textile wastewater treatment methods are inefficient and unfavorable as they are costly, consume large amount of energy, require specialized equipments and lead to the production of secondary contaminants (Adegoke and Bello, 2015; Dos Santos *et al.,* 2007; Katheresan *et al.,* 2018).

Sometimes the pollutants present in water such as pesticides, fertilizers, dyes, detergents, heavy metals, synthetic chemicals and other organic compounds are consumed by humans directly or indirectly causing them a number of health problems like cancer, hypertension, kidney damage, neurological and reproductive issues (Rai *et al.,* 2019; Wu *et al.,* 2018).

Attempts have been made by the government of India to clean River Yamuna. The Yamuna Action Plan was launched with the aim to decrease the pollutants being discharged in the river and making its water safe for various activities like irrigation, domestic water supply, bathing etc. According to an article published in Down To Earth, the Parliamentary Committee on Environment and Forests declared that the Yamuna Action Plan has failed due to its engineering-centric approach towards the problem and emphasis on construction of Sewage Treatment Plants. A study published in the International Journal of Engineering Sciences and Research Technology concluded that the even the most advance and expensive water treatment technologies were incapable in cleaning Yamuna River water.

Presently, bioremediation is one of the promising biological methods to treat polluted water as it is inexpensive, effective and eco-friendly (Dixit *et al.,* 2015; Watanabe, 2001). Bioremediation is the process in which chosen microorganisms are grown on certain pollutants to eliminate or reduce their

levels in the environment and restore it (Ali *et al.,* 2009; Mckew *et al.,* 2007; Vidali, 2001). During the process of bioremediation, the enzymes secreted by microorganisms change the structure of complex poisonous pollutants into simpler harmless compounds (Priadie, 2012). Bioremediation in water is very effective when consortium of indigenous and non-indigenous bacteria are used for decomposition of organic matter such as cellulose, lipids, and proteins as per their ability (Sastrawidana, 2008).

A bacterial consortium has higher ability of bioremediation than a single isolate (Kumar *et al.,* 2018). It has been reported that bacteria are efficient in remediating a wide range of environmental pollutants (Kour *et al.,* 2022). Bacteria are chosen over plants, algae and fungi in the process of remediation because they can grow easily, rapidly and are easy to manipulate. Owing to their ubiquitous nature, bacteria have the ability to survive in harsh and stressful environmental conditions and can utilize a large number of substrates as their carbon source (Hussain *et al.,* 2022). They can convert complex toxic compounds into non-toxic simple compounds (Azubuike *et al.,* 2016). Microbial enzymes play a pivotal role in removal of pollutants from the environment. Some of the enzymes used in bioremediation are lipase, amylase, protease, laccase, hydrolases, oxidoreductase, lignin peroxidase, dehydrogenase, oxygenase and phosphodiesterase (Ayilara and Babalola, 2023).

In 2014, Bayoumi *et al.* isolated and identified *Pseudomonas aeruginosa, P. putida* and *Bacillus cereus* which possessed the capability to decolorize textile dyes. Strains of *Enterobacter* spp. were isolated from textile effluents collected from the drains of textile industries in Bangladesh. They have the ability to decolorize Malachite green dye completely (Roy *et al.,* 2020). Pardamean *et al.,* 2021 reported that *Staphylococcus aureus* and *B. subtilis* present in sugar factory wastewater can be used as bioremediation agent as they decrease the concentration of organic matter in water. *B. paramycoides* and *Alcaligenes faecalis* strains can be used to treat hospital wastewater (Rashid *et al.,* 2022). *B. thuringiensis* and *B. toyonesis* isolated from an oil contaminated habitat produce highly active biosurfactants that can degrade hydrocarbons (Darwesh *et al.,* 2021).

When a bioremediation agent is developed from a group of liquid bacterial culture there is a decrease in the viability and performance of bacteria during the storage period (Rathore *et al.,* 2013). To prevent such problems, the bacterial cells are encapsulated to protect them from the harsh external environment (Ghosh, 2006). The commonly used materials for encapsulation are sucrose, starch, maltodextrins,

cyclodextrins, carboxymethyl cellulose and its derivatives, Arabic and agar, wax, gelatin, gluten and casein (Dubey, 2009; Suave *et al.,* 2006).

The purpose of this research is to formulate a bacterial consortium consisting of non-pathogenic bacteria having the capacity to bioremediate. These bacteria may be isolated from environmental, food or clinical sources and will be tested for the treatment of domestic sewage and textile industry pollutants discharged in Yamuna River.

REVIEW OF LITERATURE

In 2010, Misra reported an increase in the level of water pollution in river Yamuna by stating the change in water quality parameters such as BOD, COD, DO and heavy metal concentrations over a period of time at different locations. Misra also described the major causes of pollution in river Yamuna and the strict measures that can be taken to curb pollution and rejuvenate the dead river. In 2020, Sharma *et al.* measured 12 physicochemical parameters to assess the water quality of Yamuna River at Dehradun for the years 2017-2019. The study showed that temperature, TDS, hardness and total coliforms are increasing yearly with no significant changes in pH and DO, raising concern among the researchers and the concerned authorities. In 2013 Gupta *et al.* conducted a study to determine physico-chemical properties of Yamuna River water collected from nine sampling sites in Agra city. These sampling sites are Runkata, Naire Ghat, Kailash Mandir, Pohiya Ghat, Balkeshwar, Rambagh, Etmad-ud-daula, Hathi Ghat and Tajganj.

In 2020, Bhumbla *et al.* assessed the quality of water in lakes of Udaipur, Rajasthan. Thus, identifying and isolating bacteria and concluding the dominance of different coliform bacteria in these water bodies making them unfit for swimming and bathing. Ethica *et al.* (2018) justified the need to develop an efficient bioremediation agent using hydrolytic bacteria to degrade biomedical waste present in water. Pardamean *et al.* (2021) found that *Staphylococcus* sp. and *Bacillus* sp. are the two most dominant bacterial species present during the process of bioremediation and possess the ability to decrease water quality parameters like pH, BOD and COD in five days aerobically. In 2022, Rashid *et al.* isolated bacterial strains from different wastewaters like domestic, hospital, textile and pharmaceutical to remove contaminants from these wastewaters so that this water can be reused for irrigation in developing countries like Pakistan. Prabhakar *et al.* (2022) showed that the alkaliphilic *Nesterenkonia*

lacusekhoensis has the remarkable ability to decolorize more than 90% of the azo dyes present in textile wastewaters.

Besides bacteria many studies showed that fungus and algae can also be used for bioremediation of wastewater. In 2022, Muliyadi *et al*. determined that the white rot fungus *Pleurotus pulmonarius* can be used to treat textile wastewater as it has the capability to decolorize 59.45% of dyes after an incubation period of 144 hours. The present-day wastewater treatment processes are really costly and energy consuming. Therefore, microalgae-based remediation (phycoremediation) can be used as an alternative to the currently used water treatment processes (Sharma *et al.,* 2014). Algae are efficient in removing nitrate-nitrogen (NO_3-N), ammonical-nitrogen (NH_4-N), phosphorus (P), potassium (K) and reducing the biological oxygen demand (BOD) and chemical oxygen demand (COD) (Khan *et al.,* 2019). Sharma *et al.* (2020) used microalgae *Chlorella minutissima* for absorbing pollutants present in sewage wastewater. Gutiérrez *et al.* (2022) used microbial consortium with predominance of microalgae (MCPM) as an effective tool for on-site bioremediation of Mallorquín tropical swamp contaminated with sewage.

Hydrolytic bacteria can secrete enzymes that can catabolize major biomolecules such as polysaccharides, proteins, and fats present in biomass (Liu *et al.,* 2018; Singh *et al*., 2016). Hydrolytic bacteria play a significant role in bioremediation by producing enzymes such as amylase, protease, lipase, DNAse and xylanase (Ethica and Sabdono, 2017; Facchin *et al,* 2013; Karigar and Rao, 2011; Sabrina and Ethica, 2018; Vos *et al.,* 2011). It is well known that hydrolytic bacteria have the potential to improve water quality parameters (Abd El-Salam, 2010). In 2021, Darmawati *et al.* established a plate-based pathogenicity scoring system. This system can be used as a tool to select non-pathogenic hydrolytic bacteria that have the capability to act as a bioremediation agent. This system uses three agar plates namely MacConkey Agar Plate (MAP), Blood Agar Plate (BAP) and Chocolate Agar Plate (CAP). Comparatively the potential of bacterial consortium to degrade pollutants is better than individual isolates. As a result, it is crucial to formulate bacterial consortium from individual isolates in degradation studies (Dhote *et al.,* 2018; Santisi *et al*., 2015). It is important to evaluate the synergism and antagonism among the pathogenic and non-pathogenic bacteria in order to determine the capability of these bacteria to work as a bioremediation agent when mixed as consortium. To screen bacteria for synergistic

interactions cross streak method is known to be used (Hossain and Rahman, 2014; Lertcanawanichakul and Sawangnop, 2008). Overlay method is used to determine the antagonistic interactions of a non-pathogenic isolate with a pathogenic one (Ethica *et al.,* 2019).

A promising method for treatment of water is immobilization of bacteria on nanofibers which makes them easy to handle, harvest, reuse and is applied in industries. Cellulose acetate nanofibers can be used for immobilization of bacteria that can decolorize dyes such as methylene blue (Zamel and Khan, 2021). Samhan *et al.* (2017) used different non-toxic and non-polluting materials like sponge, loofah, palm, raffia and ground corn qgualh as biocarriers for immobilization of *Pseudomonas aeruginosa*. *P. aeruginosa* had been found to be very efficient in degrading crude oil in several studies (Chettri *et al.,* 2016; Das and Mukherjee, 2007; Nie *et al.,* 2016). The use of coconut coir for immobilization of microbial consortia and using this natural matrix for treatment of domestic wastewater was proposed by Memon *et al.* in 2020. In 2015, Safitri *et al.* showed that carrier materials like alginate and starch can be used to encapsulate bacterial consortium as they maintain the viability of bacterial cells thus improving their bioremediation potential when used to treat the polluted wastewater of Cimuka River.

OBJECTIVES

1) Assessment of water quality parameters of Yamuna River water.
2) Isolation and identification of bacteria that can degrade domestic sewage and textile industry pollutants.
3) Phylogenetic analysis and formulation of a non-pathogenic bacterial consortium.
4) Encapsulation of bacterial consortium on an optimal biocarrier or nanofiber.
5) Determining the efficacy of the encapsulated bacterial consortium in remediating Yamuna River water.

PROPOSED METHODOLOGY

1. Regular maintenance of aseptic conditions in the lab by fumigation, autoclaving, dry heat sterilization and filter sterilization.
2. Sampling of water samples from Yamuna River from different sites in Agra city (Bhumbla *et al*., 2020; Clesceri *et al.,* 1989; Gupta *et al.,* 2013).
3. These samples will be analyzed for various water quality parameters (APHA, 1926; Sharma *et al.,*

2020; Singh, 2020; Trivedy *et al.,* 1987)

Physical Parameters- Turbidity, Temperature, Colour, Solids (TDS, TSS, TS), Electrical conductivity.

Chemical Parameters- pH, acidity, alkalinity, hardness, DO, BOD, COD, ammonia, nitrates, potassium, sodium, chloride, phosphates.

Biological Parameters- Bacteria.

4. Isolating bacteria from different samples of Yamuna River water by using traditional microbial methods or metagenomic analysis approach (Hesnawi *et al.,*2014; Purwaningrum *et al.,* 2021).
5. Screening of enzyme producing bacteria by using agar plate-based activity assays (Ethica *et al.,* 2018; Sánchez-Porro *et al.,* 2003).
6. Testing the pathogenicity of selected bacteria by following the plate-based pathogenicity scoring system consisting of MacConkey Agar Plate (MAP), Blood Agar Plate (BAP) and Chocolate Agar Plate (CAP) (Darmawati *et al.,* 2021).
7. Molecular identification of selected bacteria by 16s rRNA gene sequencing and phylogenetic analysis for tree construction (Ethica and Sabdono, 2021; Iskandar *et al.,*2021; Radjasa *et al*., 2007).
8. Determining the degradation performance of the selected bacterial isolates on domestic sewage and textile pollutants by analyzing the changes in physico-chemical parameters, reduction in heavy metal concentration and decolorization of dyes (Chaurasia *et al*., 2022; Gutiérrez *et al.,* 2022; Prabhakar *et al*.,2022; Sharma *et al.,* 2020).
9. Formulating a bacterial consortium based on their efficiency to degrade pollutants, acceleration of the bioremediation process, antagonistic and synergistic interactions. Efficiency to degrade pollutants can be analyzed by the improvement in physico-chemical parameters. Acceleration of the bioremediation process can be evaluated statistically. Antagonistic interactions can be determined by Overlay method and synergistic interactions by Cross Streak method (Dhall *et al.,* 2012; Ethica *et al.,* 2019; Safitri *et al.,* 2015).
10. Encapsulating bacterial consortium on a suitable carrier or nanofiber by using one of these previously reported encapsulating techniques like freeze drying, electrospinning or adhesion techniques like adsorption (Memom *et al.,* 2020; Safitri *et al.,* 2015; Samhan *et al.,* 2017; Van *et al.,*

1990; Zamel and Khan, 2021).

11. Using this encapsulated bacterial consortium for bioremediation of water supply of Yamuna River.

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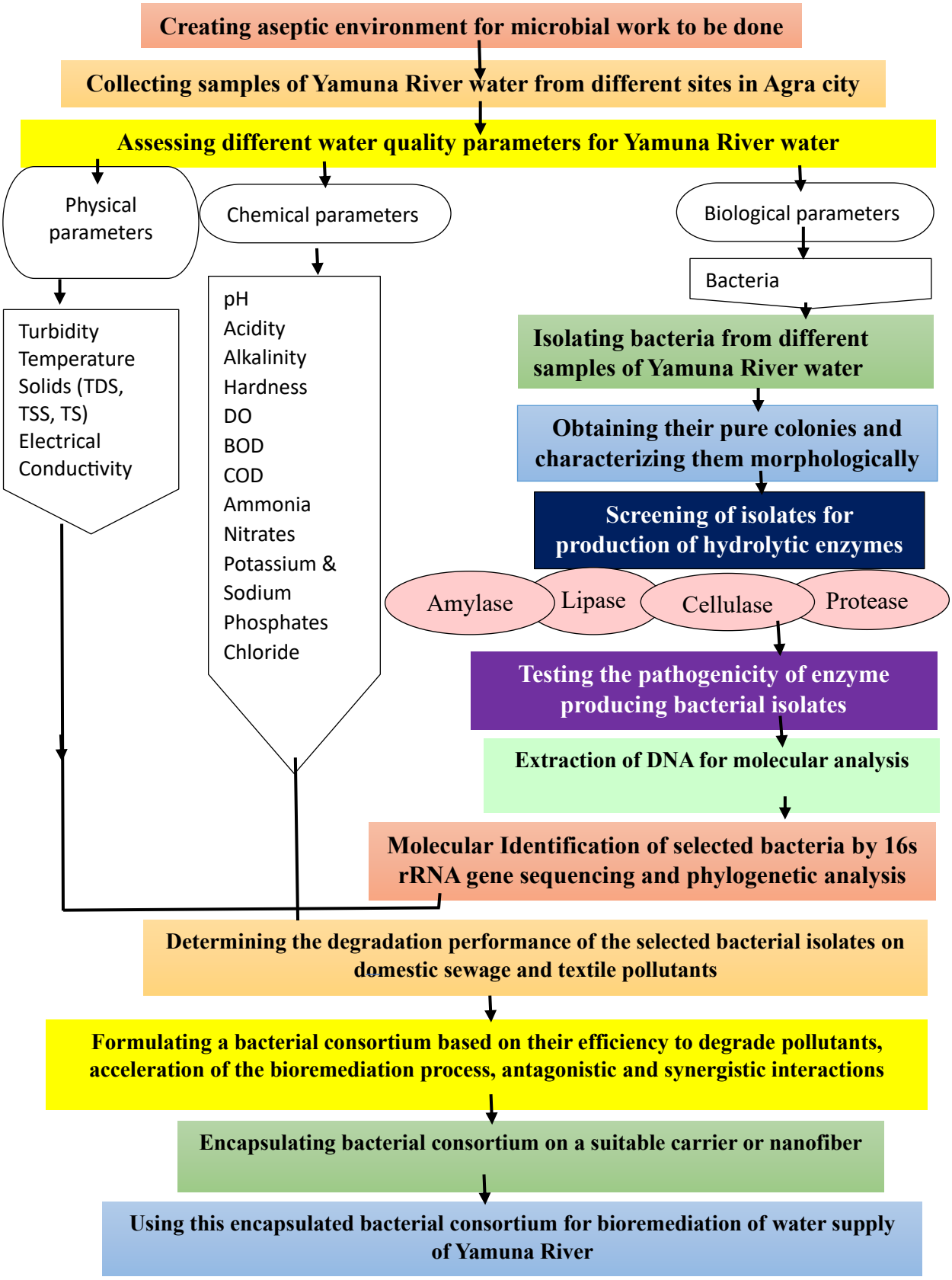
APPENDIX

PROPOSED WORK PLAN


Creating aseptic environment for microbial work to be done
Collecting samples of Yamuna River water from different sites in Agra city
Assessing different water quality parameters for Yamuna River water
Physical parameters
Chemical parameters
Biological parameters
Bacteria
Turbidity
Temperature
Solids (TDS, TSS, TS)
Electrical Conductivity
pH
Acidity
Alkalinity
Hardness
DO
BOD
COD
Ammonia
Nitrates
Potassium & Sodium
Phosphates
Chloride
Isolating bacteria from different samples of Yamuna River water
Obtaining their pure colonies and characterizing them morphologically
Screening of isolates for production of hydrolytic enzymes
Amylase
Lipase
Cellulase
Protease
Testing the pathogenicity of enzyme producing bacterial isolates
Extraction of DNA for molecular analysis
Molecular Identification of selected bacteria by 16s rRNA gene sequencing and phylogenetic analysis
Determining the degradation performance of the selected bacterial isolates on domestic sewage and textile pollutants
Formulating a bacterial consortium based on their efficiency to degrade pollutants, acceleration of the bioremediation process, antagonistic and synergistic interactions
Encapsulating bacterial consortium on a suitable carrier or nanofiber
Using this encapsulated bacterial consortium for bioremediation of water supply of Yamuna River